Pharmacognostic And Formulation Studies of An Antimicrobial Gel of Calotropis Gigantea

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***Abstract*—This project focuses on the pharmacognostic evaluation and formulation of an antimicrobial gel derived from Calotropis gigantea for potential therapeutic and skincare applications. The study involves systematic investigation of the plant's morphological, microscopic, and physicochemical characteristics to ensure accurate identification and quality assessment of the raw material. Extracts obtained from Calotropis gigantea were incorporated into a suitable gel base to develop a stable and effective antimicrobial formulation. The antimicrobial activity of the formulated gel was assessed using in vitro assays against selected pathogenic microorganisms to determine its inhibitory potential. Additionally, physicochemical parameters such as pH, spread ability, viscosity, and stability were evaluated to ensure the gel's suitability for topical use. Skin irritancy studies were also conducted to ensure its safety. Preliminary findings indicate that the formulated gel exhibits promising antimicrobial activity, supporting the traditional medicinal use of Calotropis gigantea. The results suggest that this plant-based gel may serve as a natural and effective alternative to conventional antimicrobial agents. Overall, the study highlights the potential of Calotropis gigantea as a valuable resource in the development of safe, effective, and sustainable topical antimicrobial formulations.**

***Index Terms*— Calotropis gigantea; Antimicrobial gel; Pharmacognostic evaluation; Formulation studies; Topical application.**

I. INTRODUCTION

The use of medicinal plants for maintaining health and treating diseases dates back to ancient civilizations. Herbal preparations continue to play an important role, especially in developing regions, due to their natural origin, cost-effectiveness, and relatively low incidence of side effects. In recent years, there has been increasing interest in plant-based formulations for dermatological conditions such as skin infections, wounds, burns, and inflammatory disorders. Medicinal plants contain a wide range of bioactive compounds that provide antimicrobial, antioxidant, anti-inflammatory, and wound healing properties. Compared to synthetic formulations, which may cause irritation, toxicity, or allergic reactions in some cases, herbal preparations are generally considered more compatible with biological systems. According to the Drugs and Cosmetics Act, topical formulations are dosage forms intended to be applied to the body for cleansing, protection, or therapeutic effects. In this context, medicinal plants such as Calotropis gigantea have gained significant attention due to their broad pharmacological potential.

Herbal medicine has been an integral part of traditional systems of medicine including Ayurveda, Siddha, Unani, Chinese, Egyptian, and Greek practices for centuries. According to the World Health Organization (WHO), herbal medicines include herbs, herbal materials, herbal preparations, and finished herbal products that contain plant parts or plant-derived bioactive constituents. These formulations are

widely accepted due to their perceived safety, affordability, and compatibility with human physiology. They are rich in secondary metabolites such as alkaloids, flavonoids, terpenoids, glycosides, tannins, and phenolic compounds, which are responsible for their diverse pharmacological activities.

Topical drug delivery refers to the application of drugs directly onto the skin to achieve localized therapeutic effects. This route is particularly effective for managing skin-related conditions such as infections, inflammation, acne, and wounds, as it minimizes systemic exposure and associated side effects. Common topical formulations include gels, creams, ointments, and lotions. Among these, gels are often preferred because they are non-greasy, easily spreadable, cosmetically acceptable, and provide faster drug release. Ointments and lotions, on the other hand, are more suitable for occlusive or moisturizing effects. Overall, topical delivery systems offer the advantage of direct drug action at the site of application, thereby improving therapeutic efficiency while reducing systemic toxicity. In contrast, transdermal drug delivery systems are designed to deliver drugs into systemic circulation, whereas topical systems primarily act locally with minimal systemic absorption.

The skin is the largest organ of the human body and acts as the first line of defense against environmental hazards such as microbes, chemicals, ultraviolet radiation, and physical injuries. It consists of three main layers: epidermis, dermis, and subcutaneous tissue. The epidermis forms the outer protective barrier, the dermis contains blood vessels, hair follicles, sweat glands, and sebaceous glands, while the subcutaneous layer provides insulation and cushioning. The slightly acidic pH of the skin (approximately 4–6) plays a significant role in inhibiting microbial growth and maintaining the skin barrier function. Together, these layers regulate temperature, maintain hydration, and support immune defense mechanisms, making the skin an important target for topical therapeutic interventions.

Herbal antimicrobial gels are semi-solid dosage forms containing plant extracts with antibacterial, antifungal, and wound-healing properties. These formulations are designed to reduce microbial load, prevent infections, soothe inflamed tissues, and enhance the wound healing process. They also offer advantages such as uniform drug distribution, improved patient compliance, enhanced skin absorption, and better acceptability compared to conventional formulations. Calotropis gigantea, commonly known as Giant Milkweed, is a medicinal plant widely used in traditional medicine for the treatment of skin diseases, wounds, inflammation, and infections. It contains important phytoconstituents such as flavonoids, cardenolides, tannins, and triterpenoids, which are responsible for its antimicrobial and anti-inflammatory activities. Therefore, developing a topical gel using Calotropis gigantea extract provides a promising natural approach for managing microbial skin conditions effectively and safely.

Calotropis gigantea (L.) W.T. Aiton, also known as Crown Flower or Giant Milkweed, belongs to the family Apocynaceae. It is a large evergreen shrub that can grow up to 4 meters in height and produces abundant milky latex. The plant is characterized by opposite, broad, leathery leaves, waxy star-shaped flowers, inflated fruit follicles, and seeds with silky hairs that aid dispersal. It is widely distributed across tropical and subtropical regions including India, Sri Lanka, Bangladesh, Nepal, Southeast Asia, Africa, and parts of Arabia. It commonly grows in dry wastelands, roadsides, and sandy soils, and is also cultivated as an ornamental and religious plant.

Phytochemical studies have reported the presence of more than 150 bioactive compounds in Calotropis gigantea, including cardiac glycosides (calotropin, uscharin, calotoxin), flavonoids (quercetin, kaempferol, isorhamnetin), terpenoids (giganteol, beta-sitosterol), triterpenes (alpha and beta amyrin), alkaloids (calotropaine), phenolics, and latex-based constituents. These compounds collectively contribute to its wide range of pharmacological activities. The plant exhibits antimicrobial, anti-inflammatory, antioxidant, analgesic, wound healing, anticancer, larvicidal, insecticidal, and antipyretic properties. In particular, its latex and leaf extracts have shown strong antimicrobial activity against both Gram-positive and Gram-negative bacteria as well as fungal organisms, supporting its use in topical formulations.

Traditionally, Calotropis gigantea has been used in Ayurveda, Siddha, and Unani systems of medicine. The latex is applied externally for skin diseases, fungal infections, and joint pain, while leaves are used for treating swelling and inflammation. The root bark is used as an expectorant and purgative, and flowers are

used for digestive disorders. The plant is also applied to wounds, cuts, boils, and ulcers due to its antiseptic properties. Leaf poultices are commonly used for infected wounds, further supporting its relevance in antimicrobial therapy.

The present study is based on the development and evaluation of an antimicrobial gel using Calotropis gigantea leaf extract. Although the plant has been extensively studied for its pharmacological and phytochemical properties, most of the existing research is limited to crude extract screening and basic antimicrobial assays. There is comparatively less focus on the development of a stable, standardized, and patient-friendly topical gel formulation. In addition, comprehensive evaluation involving pharmacognostic authentication, detailed phytochemical standardization, and full physicochemical characterization of gel parameters such as pH, viscosity, spread ability, drug content, and stability is still limited. Another gap is the insufficient integration of traditional pharmacognostic knowledge with modern formulation science to ensure reproducibility and quality control. Moreover, comparative studies with standard antimicrobial formulations and long-term stability assessments are not adequately explored. Therefore, there is a clear need to develop a well-standardized antimicrobial gel of Calotropis gigantea leaf extract with thorough pharmacognostic, phytochemical, physicochemical, and antimicrobial evaluation to establish its safety, effectiveness, and potential as a topical therapeutic system.

Fig 1: Erukku flower, Erukku plant

II. MATERIALS AND METHODS

2.1 Authentication

Leaves of Calotropis gigantea was collected from the tree present in the region of Thrissur Kerala (Holy grace academy of pharmacy). And it is authenticated from Kerala agricultural university, Mannuthy, Thrissur.

Fig 2: Collection of plant leaves

2.2 Macroscopic and Microscopic Evaluation of Calotropis gigantea Leaf

The leaves are simple and exstipulate, arranged in an opposite and decussate pattern. They are ovate to obovate in shape and measure approximately 12–20 cm in length and 5–10 cm in width. The apex is acute to shortly acuminate, while the base is cordate and sessile. The leaf margin is entire, and the texture is thick, leathery, and fleshy. The venation is reticulate in nature, confirming typical dicot leaf characteristics.

Fig 3: Macroscopy of leaf

Microscopic examination of the transverse section of the leaf shows a dorsiventral structure with distinct upper and lower epidermis, each composed of a single layer of cells covered by a thick cuticle. Anomocytic stomata are present, with a higher density on the lower epidermis. Multicellular covering trichomes are also observed on the epidermal surface.

The mesophyll is differentiated into a well-defined palisade parenchyma (1–2 layers) and spongy parenchyma with prominent intercellular spaces.

Numerous laticifers (latex ducts) are distributed throughout the mesophyll and vascular regions. The vascular bundles are collateral and closed, surrounded by a parenchymatous bundle sheath. Occasional calcium oxalate crystals are also present within the tissue structure.

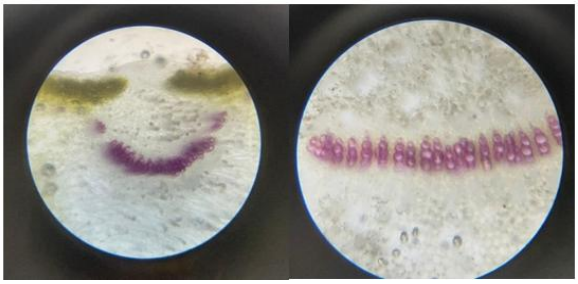

Fig 4: T.S. of Calotropis gigantea

These macroscopic and microscopic characteristics collectively confirm the identity and diagnostic features of Calotropis gigantea leaf material used in the study.

2.2 Preparation of extract

The plant leaves were then washed, dried, and powdered. Then it has undergone extraction using maceration technique. The Fig shows the drying, powdering and maceration of powdered material in ethanol (95 %). And the Fig 06 shows the filtration, evaporation and collection of final extract.

Fig 5: Drying, size reduction, powder, maceration

Fig 6: Filtering using muslin cloth, filter paper, evaporation, herbal extract

2.3 Preliminary phytochemical investigation

Preliminary phytochemical screening of Calotropis gigantea leaf extract was carried out to identify the presence of major bioactive constituents using standard qualitative methods. For this, 0.5 g of the extract was dissolved in distilled water and subjected to different chemical tests. Alkaloids were detected using Dragendorff's and Mayer's tests. Glycosides were identified using Keller-Killiani and Legal's tests. Flavonoids were confirmed by Shinoda test (magnesium ribbon and concentrated HCl) and alkaline reagent test using NaOH solution. Tannins were tested using ferric chloride and lead acetate tests. Terpenoids and steroids were identified using Salkowski test and Liebermann-Burchard test involving chloroform, acetic anhydride, and concentrated sulfuric acid. Phenolic compounds were confirmed using ferric chloride and lead acetate reactions. These tests confirmed the presence of multiple phytochemical groups responsible for antimicrobial and therapeutic activity of the plant.

2.4 Preparation of Antimicrobial Gel

An antimicrobial gel was formulated using Calotropis gigantea extract due to its antimicrobial, anti-inflammatory, and wound-healing properties. Carbopol 940 was used as the gelling agent, propylene glycol and glycerin as humectants and penetration enhancers, methylparaben as preservative, and triethanolamine for pH adjustment. The extract was incorporated by levigation to ensure uniform dispersion, producing a stable and easily spreadable topical gel.

Procedure:

Carbopol 940 (0.7 g) was dispersed in 70 ml purified water and allowed to swell for 24 hours. Triethanolamine was added dropwise to adjust pH to 6–7, forming the gel base. A mixture of propylene glycol, glycerin, and methylparaben was added, followed by disodium EDTA and Calotropis gigantea extract. Rose oil was incorporated, and the formulation was homogenized to obtain a uniform gel.

Table 1: Ingredients with their functions

INGREDIENTS	FUNCTION
Calotropis gigantea extract	API
Carbopol 940	Gelling agent
Glycerin	Humectant
Propylene Glycol	Penetration enhancer

Disodium EDTA	Chelating agent
Triethanolamine	pH adjuster
Methylparaben	Preservative
Rose oil	Perfuming agent
Purified water	Vehicle

2.5 Formulation and evaluation of Gel

The gel was formulated by dispersing 0.7 g of Carbopol 940 in 70 ml of purified water and allowing it to hydrate for 24 hours. After hydration, a triethanolamine (TEA) solution (1 ml TEA in 9 ml water) was added dropwise with continuous stirring until the pH reached 6.0–7.0, forming the gel base. A separate mixture of propylene glycol (5 ml), glycerin (5 ml), and methylparaben (0.18 g) was heated until complete dissolution and then cooled before being incorporated into the gel base along with disodium EDTA (0.05 g). The extract phase was prepared by dissolving 2 g of Calotropis gigantea extract in a mixture of propylene glycol and glycerin (1:1) and triturated to ensure uniformity. This extract solution was then added to the gel base with gentle stirring or homogenization. Finally, rose oil was added as a perfuming agent, and the volume was made up to 100 g with purified water to obtain a uniform gel formulation.

Table 2: Formulation of gel

INGREDIENT	F1	F2	F3	F4
Calotropis gigantea extract (g)	0.5	1.0	1.5	2.0
Carbopol 940 (g)	0.7	0.7	0.7	0.7
Glycerin (ml)	10.0	10.0	10.0	10.0
Propylene glycol (ml)	10.0	10.0	10.0	10.0
Disodium EDTA (g)	0.05	0.05	0.05	0.05
Triethanolamine (ml)	1.0	1.0	1.0	1.0
Methylparaben (g)	0.18	0.18	0.18	0.18
Rose oil (ml)	0.1	0.1	0.1	0.1
Purified water (ml)	77.47	76.97	76.47	75.97

Table 3: Evaluation of Herbal Antimicrobial Gel

Parameter	Method	Observation/Inference
Colour	Visual inspection	Characteristic color observed
Odour	Smelling	Pleasant/characteristic odour
Texture	Touch	Smooth and uniform
State	Visual	Semisolid
Consistency	Applied on skin	Good consistency
Homogeneity.	Visual inspection	No lumps or aggregates
pH	Digital pH meter (1 g gel in 100 ml water)	Within skin pH range (6–7)
Spreadability	Slide method (S = M × L / T)	Good spreadability
Washability	Shake flask method	Easily washable
Viscosity	Brookfield viscometer (12 rpm)	Suitable viscosity
Greasiness	Gravimetric method (hexane extraction)	Non-greasy nature
Antimicrobial Activity	Agar well diffusion method	Zone of inhibition observed

III. RESULT AND DISCUSSION

3.1 Preliminary phytochemical investigation

The prepared extract was subjected to qualitative phytochemical screening to identify the presence of major bioactive constituents. For the analysis, 0.5 g of extract was dissolved in distilled water and tested using standard procedures.

Alkaloids were detected using Dragendorff's test and Mayer's test, glycosides by Keller-Killiani and Legal's test, flavonoids by Shinoda and alkaline reagent test, tannins by ferric chloride and lead acetate tests, terpenoids and steroids by Salkowski and Liebermann-Burchard tests, and phenolic compounds using ferric chloride and lead acetate reactions. These tests confirmed the presence of multiple phytochemical groups responsible for antimicrobial and therapeutic activity.

Fig 7: Detection of alkaloids

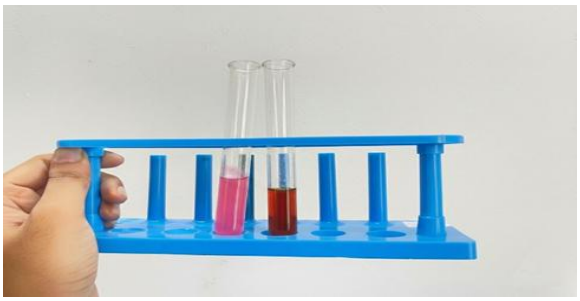

Fig 8: Detection of glycosides

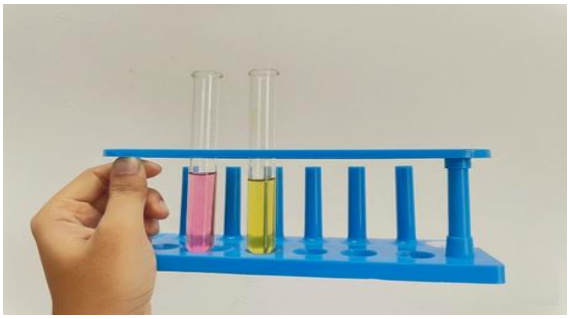

Fig 9: Detection of flavonoids

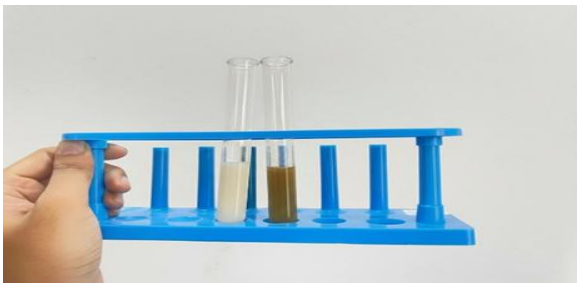

Fig 10 Detection of tannins

3.2 Preparation of antimicrobial gel

A gel base using carbopol 940 was made. The Fig 07 shows the incorporation of plant extract into the gel base to form the antimicrobial gel. It is gently agitated using an electric homogenizer. Figure 11

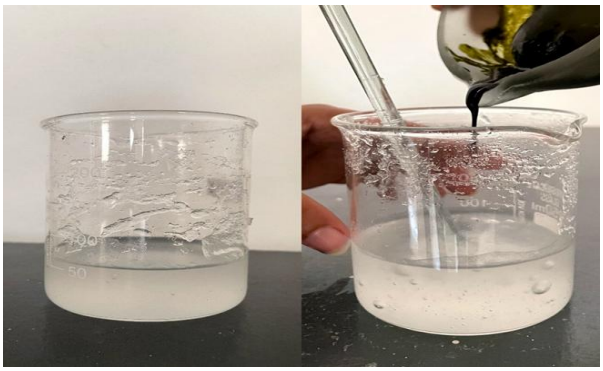

Fig 11: Incorporation of extract into gel base

3.3 Formulation of gel

Four different formulations are made by adjusting the quantity of the extract.

Fig 12: Formulation 1 and Formulation 2

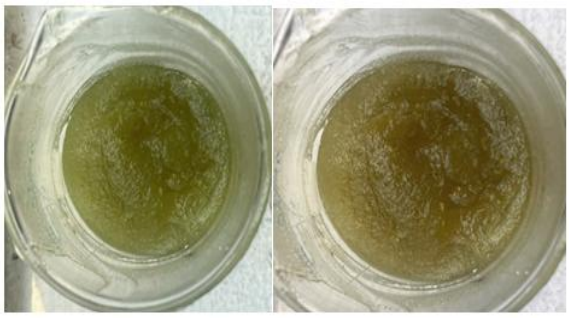

Fig 13: Formulation 3 and Formulation 4

The gel formulation was prepared using a standard procedure. Initially, 0.7 g of Carbopol 940 was accurately weighed and dispersed in 70 ml of purified water, allowing it to hydrate for 24 hours. After complete hydration, a triethanolamine (TEA) solution was prepared by mixing 1 ml of TEA with 9 ml of water. This solution was added dropwise to the hydrated Carbopol under slow stirring until the pH reached 6.0–7.0, resulting in the formation of a gel base. Separately, propylene glycol (5 ml), glycerin (5 ml), and methylparaben (0.18 g) were heated in a water bath until the methylparaben dissolved completely, and the mixture was then cooled. The cooled mixture was incorporated into the gel base along with disodium EDTA (0.05 g).

For the extract phase, Calotropis gigantea extract was dissolved in a mixture of propylene glycol and glycerin (1:1 ratio) and triturated using a mortar and pestle to obtain a uniform solution. This extract solution was then gradually added to the gel base with gentle stirring or homogenization to avoid disruption of the gel structure. Rose oil was added as a perfuming

agent, and the final volume was adjusted to 100 g with purified water, followed by thorough mixing.

Four formulations (F1–F4) were prepared with varying concentrations of Calotropis gigantea extract (0.5 g, 1.0 g, 1.5 g, and 2.0 g, respectively), while all other components Carbopol 940 (0.7 g), glycerin (10 ml), propylene glycol (10 ml), disodium EDTA (0.05 g), triethanolamine (1 ml), methylparaben (0.18 g), and rose oil (0.1 ml) remained constant. The quantity of purified water was adjusted accordingly (77.47 ml, 76.97 ml, 76.47 ml, and 75.97 ml for F1 to F4, respectively) to maintain the final weight at 100 g.

3.4 Physical evaluation of gel

The formulated herbal antimicrobial gel was further evaluated using various physical parameters. The colour of the gel was assessed by visual inspection. The odour was determined and found to be characteristic of the formulation. The texture, referring to the surface characteristics and overall appearance, was also examined visually. The physical state of the gel was observed to determine its form and uniformity. Additionally, the consistency of the formulation was evaluated by gently rubbing a small quantity of the gel between the fingers to assess its smoothness and spread ability.

Table 4: physical evaluation of gel

Ingredients	F1	F2	F3	F4
Colour	Faint Green	Pale Green	Intense Green	Deep Green
Odour	Pleasant Odour	Pleasant Odour	Pleasant Odour	Pleasant Odour
State	Semisolid	Semisolid	Semisolid	Semisolid
Consistency.	Gelly	Gelly	Gelly	Gelly
Texture	Non-Slimy	Non-Slimy	Non-Slimy	Non-Slimy

Fig 14: Physical evaluation of gel

3.5 pH of Anti-microbial gel

A digital pH meter can be used to measure the pH of the generated gel compositions. After dissolving 1 gram of gel in 100 millilitres of distilled water, it is left for two hours. Each formulation's pH should be measured three times, and the average results are computed.

Table 5: pH of Anti-microbial gel

Parameter	F1	F2	F3	F4
pH	5.5	5.8	6.0	6.8

3.6 Spreadability test

Spreadability of the gel was determined using a wooden block apparatus fitted with a pulley. Approximately 2 g of the gel was placed on a ground glass slide and sandwiched between it and a second slide attached to a hook. An 80 g weight was applied to the upper slide using a string, and the time (in seconds) required for the slide to move a distance of 7.5 cm was recorded. Spreadability was calculated using the formula:

$S = (M \times L) / T$

where S is spreadability, M is the weight applied, L is the distance moved (7.5 cm), and T is the time taken in seconds.

Table 6: Spreadability

Parameter	F1	F2	F3	F4
Spreadability	Not good	Not good	Good	Not good

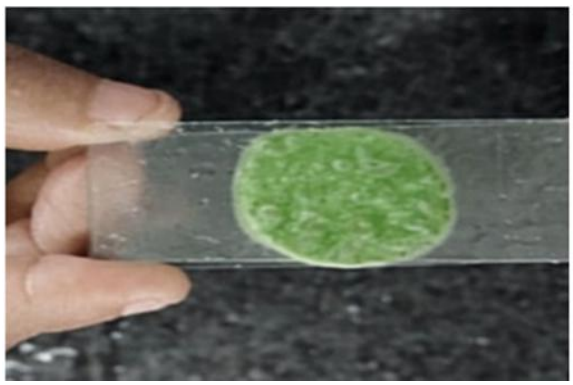

Fig 15: Spreadability test

3.7 Washability

Shake Flask Method:

Prepare the gel product sample according to the manufacturer's instructions. Apply a controlled amount of the gel product to a surface substrate (e.g., glass plate). Place the substrate with the gel product in the shake flask apparatus. Add a measured amount of water (and surfactant solution, if required) to the flask. Close the flask and shake it for a specified time (e.g., 30 seconds to 1 minute) at a controlled temperature (e.g., 25°C). After shaking, rinse the substrate with water to remove any remaining gel product. Measure the amount of gel product removed from the substrate using a suitable analytical technique (e.g., spectrophotometry, chromatography). Repeat the test multiple times to ensure reproducibility.

Table 7: Washability

Parameter	F1	F2	F3	F4
Washability	Not good	Good	Easily washable (Good)	Good

3.8 Viscosity

The viscosity of gel was determined using Brookfield Viscometer with 12 rpm spindle. Insert the spindle into sample, rotate spindle and wait for the reading stabilize and finally record the reading.

Table 8: viscosity

Parameter	F1	F2	F3	F4
Viscosity (cp)	9100	9550	10300	10050

3.9 Greasiness

Gravimetric analysis:

Accurately weigh a representative sample of the gel. Add a known volume of hexane to the gel sample and mix thoroughly to extract the greasy components. Separate the liquid (hexane extract) from the gel using a suitable method like filtration or centrifugation. Carefully evaporate the hexane from the extract in a pre-weighed container using a gentle heat source like a water bath. Once the solvent is completely evaporated, weigh the container to determine the mass of the extracted grease residue.

Table 9: Greasiness

Parameter	F1	F2	F3	F4
Greasiness	Non greasy	Non greasy	Non greasy	Non greasy

3.10 Anti-microbial test

Antimicrobial activity was evaluated using the agar well diffusion method. Nutrient agar medium was prepared, sterilized by autoclaving, and poured into sterile Petri plates, where it was allowed to solidify. The test microorganisms were then uniformly swabbed onto the surface of the agar plates.

For sample preparation, an appropriate quantity of the sample was weighed and dissolved in a suitable solvent such as methanol or distilled water to obtain the sample solution. Wells were created in the agar plates using a sterile corn borer, and a measured volume of the sample solution was introduced into each well. A standard antibiotic was used as a positive control, while the solvent served as a negative control. The plates were then incubated at 37°C for 24 hours.

Table 10: anti-microbial test

Test Organism.	Sample (mm)	Standard Drug (Streptomycin 1000 ppm) (mm)	Test Method
Staphylococcus aureus (NCIM 2127).	25 mm	31 mm	CKL/MB/MOA-044

IV. CONCLUSION

The current work concentrated on the pharmacognostic assessment and creation of an antimicrobial gel employing Calotropis gigantea, a plant rich in bioactive substances with antibacterial and wound-healing qualities, including cardiac glycosides, flavonoids, tannins, and alkaloids. The purity of the plant material was verified by authentication using microscopic, macroscopic, and

phytochemical analyses. The extract was effectively added to gel formulations (F1–F4), which demonstrated high stability and patient acceptability with respect to physicochemical qualities such as pH, viscosity, spread ability, washability, and consistency. F3 was shown to be the best formulation among them. Its efficacy as a topical antibacterial agent was supported by the antimicrobial investigation utilizing the agar well diffusion method, which demonstrated substantial action against Staphylococcus aureus. Overall, the study validates the traditional use of Calotropis gigantea by concluding that it can be turned into a stable, safe, and effective herbal antibacterial gel. This formulation offers tremendous future potential in light of issues like antibiotic resistance and the rising desire for natural medicines. Its effectiveness can be increased and its uses in dermatological problems including wounds, acne, and infections expanded with more research incorporating advanced standardization (HPTLC, HPLC), stability testing, clinical evaluation, and innovative delivery systems like nano-gels.

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